The Efficiency of SNP-Based Microarrays in the Detection of Copy-Neutral Events at 15q11.2 and 11p15.5 Loci

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Abstract

Prader–Willi, Angelman, Beckwith–Wiedemann, and Russell–Silver are imprinting syndromes. In this study, we aimed to compare the efficiency of single nucleotide polymorphism (SNP) microarray analysis with methylation-specific Multiplex ligation-dependent probe amplification (MS-MLPA) in the detection of uniparental disomy in these syndromes. The patient samples with regions of loss of heterozygosity (LOH), covering 15q11.2 and 11p15.5 critical loci, were analyzed with MS-MLPA to demonstrate the efficiency of SNP microarray in the detection of uniparental disomy (UPD). In a total of seven patients, LOH covering 15q11.2 and 11p15.5 critical loci was detected. Two (28.6%) of these seven patients showed aberrant methylation (suggesting UPD) in MS-MLPA. SNP microarray is a useful tool in the detection of LOH; however, it should be used with caution, since false-positive or false-negative LOH results can be obtained. Although methylation analysis is recommended as the first tier test in the diagnosis of most of the imprinting disorders, combining methylation analysis with SNP microarray can enhance our evaluation process.

Keywords
- SNP microarray
- loss of heterozygosity
- uniparental disomy

Introduction

Genomic imprinting is an epigenetic inheritance phenomenon in which the gene expression is regulated according to the parent of origin. In most of the genes, both copies are functional. However, in imprinted genes, only one copy is functional and the other is silenced depending on the parental origin. This parent-dependent silencing of a gene results from an epigenetic marking mechanism, which mainly uses cytosine methylation at CpG dinucleotides during gametogenesis. This imprint is carried for a generation until the individual rearranges it at his/her gamete production.[1,2]

In humans, more than 100 genes have been shown to be involved in genomic imprinting, and these genes are listed in the National Center for Biotechnology Information database, geneimprint.com (http://www.geneimprint.com).[3] Imprinting problems can cause two functional or two silenced copies of a gene. Since many imprinted genes are associated with growth and metabolism, the main clinical consequences of imprinting disorders are associated with overgrowth or undergrowth, developmental delay, intellectual disability, and distinctive behavioral phenotypes. Furthermore, aberrant imprinting can also lead to multigenic disorders and cancer.

Imprinted genes tend to be in close proximity; thus, certain loci have been identified. In these loci, imprinting control regions (ICRs) regulate the expression of the imprinted genes in their region. This regulation is done by coordination of DNA methylation, by changing chromatin structure, and by posttranslational histone modifications.[4]

The pathogenic process that alters normal imprinting can be associated with several genetic (sequence variants in imprinted genes, copy number variants [CNVs] covering

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imprinted genes, and uniparental disomy [UPD]) or epigenetic mechanisms (aberrant methylation/epimutations).[3] Since the inheritance patterns of these four pathogenic mechanisms and recurrence risks are different, the identification of the underlying defect is important for accurate genetic counseling.

Among these 100 imprinted loci, the two loci, 15q11.2 and 11p15.5, are much more pronounced in terms of frequency and clinical importance. Four well-known imprinting disorders, Prader–Willi syndrome (PWS), Angelman syndrome (AS), Beckwith–Wiedemann syndrome (BWS), and Russell–Silver syndrome (RSS) are associated with these critical regions.

PWS (OMIM #176270) is mainly characterized by severe hypotonia in infancy and morbid obesity in childhood. Mental and motor retardation with distinct behavioral phenotype, short stature, hypogonadism, and dysmorphic facial features are the other common features of the disease.

AS (OMIM #105830) is the other disease which is associated with the defects in the 15q11.2 region. AS is characterized by microcephaly, seizures, mental and motor retardation, ataxia, and a unique inappropriate happy behavior.

BWS (OMIM #130650) is an overgrowth disorder. It is usually suspected in infancy with macrosomia and hypoglycemia. The disease causes macroglossia, hemihyperplasia, omphalocele, embryonal tumors, visceromegaly, and renal abnormalities.

RSS (OMIM #180860) is an undergrowth disorder, clinically starting at the intrauterine period. Patients present with growth deficiency with normal head circumference, proportionately short stature, and typical triangular face. The two chromosomal regions, 11p15.5 and 7q, are associated with RSS.

The 15q11.2 locus harbors the five paternally expressed imprinted genes (Makorin 3 [*MKRN3*], Mage-like 2 [*MAGEL2*], necdin [*NDN*], small nuclear ribonucleoprotein polypeptide N [*SNRPN*]), a cluster of paternally expressed noncoding RNA [snoRNAs] and two maternally expressed imprinted genes (ubiquitin-protein ligase E3A [*UBE3A*] and ATPase class V type10A [*ATP10A*]). When the loss of expression of paternally inherited genes (gain of methylation [GOM] in paternally imprinted genes) leads to PWS, the loss of expression of maternally inherited genes (loss of methylation [LOM] in paternally imprinted genes) leads to AS.[5]

At 11p15.5 locus, the two clusters of imprinted genes, long intergenic noncoding RNA (*H19*)/insulin-like growth factor II (*IGF2*) (imprinting center 1 [IC1]) and KCNQ1-overlapping transcript 1 (*KCNQ1OT1*) (imprinting center 2 [IC2]), are associated with BWS and RSS.[6] When the GOM of IC1 and LOM of IC2 are associated with BWS, LOM of IC1 is associated with RSS.

The frequency and the order of genetic and epigenetic mechanisms that cause these imprinting disorders are summarized in ►**Table 1**.

Generally, DNA methylation analysis is recommended as the first tier test in the diagnosis of PWS, AS, BWS, and RSS, since it will detect the consequent pathogenic effect of the above-mentioned genetic mechanisms, aberrant methylation. This methylation analysis can be done with "methylation-sensitive RFLP," "methylation-specific polymerase chain reaction (PCR)," methylation-sensitive high-resolution melting curve analysis, and "methylation-specific multiplex ligation-dependent probe amplification" (MS-MLPA).[7] In clinical practice, MS-MLPA is the preferred method, since it does not require special infrastructure and it does not include laborious and complex techniques such as Southern blot. However, MS-MLPA only analyses a limited region with a limited number of probes, incidental sequence changes can cause false-positive/negative results, it only shows the status of the targeted CpG sites and no discrimination can be made between UPD and imprinting defects.

With the use of single nucleotide polymorphism (SNP)-based chromosomal microarray, in addition to the detection of the CNVs, it also became possible to uncover copy number neutral events, such as loss of heterozygosity (LOH) originating from UPD. SNP microarray has also the advantage to determine the parental origin, breakpoints and the sizes of abnormal fragments, and the ratio of mosaicism, in both the targeted regions and in the entire genome.[8] In clinical practice, because of these, SNP microarrays have started to be a preferred method in the patients suspected of imprinting disorders.[9]

In this study, we aimed to compare the efficiency of SNP microarray analysis with MS-MLPA in the detection of copy number neutral events, UPD/LOH, in PWS, AS, BWS, and RSS.

Materials and Methods

Patient Selection

The patients were accepted for clinical problems such as congenital anomalies (CA), developmental disabilities (DD), hypotonia, dysmorphism (PWS/AS), and hemihypertrophy (BWS/RSS).

The patients suspected of PWS, AS, BWS, and RSS were first analyzed with SNP-based microarray. If microdeletions associated with these diagnoses were detected, no additional test was planned; however, if LOH was detected, MS-MLPA was performed in the next step.

SNP-Based Microarray Analysis

Genomic DNA was isolated from peripheral blood samples according to the manufacturer's protocols. For SNP microarray analysis, CytoScan Optima Array Kit from Affymetrix (Thermo Fisher Scientific, Santa Clara, California, United States) was used. All microarray procedures were performed using GeneChip Hybridization Oven 645, GeneChip Fluidics Station 450, and GeneChip Scanner 3000 from Affymetrix. All microarray data were analyzed with Chromosome Analysis Suite (ChAS) 3.1 from Affymetrix (Thermo Fisher Scientific), using GRCh37/hg19 libraries.

The patient evaluation was started with CNV analysis. If no pathogenic CNVs were found, LOH analysis was done. In the LOH analysis, the LOH regions covering less than 50 markers were filtered out.

Multiplex Ligation-Dependent Probe Amplification

For the MS-MLPA analysis, SALSA MS-MLPA Probemix ME028 Prader-Willi/Angelman (MRC-Holland, Amsterdam, The Netherlands) and SALSA MS-MLPA Probemix ME030 BWS/RSS (MRC-Holland, Amsterdam, The Netherlands) were

Table 1 Etiologies, frequencies, resulting genotypes, and methylation status of the disorders

Prader–Willi syndrome					Angelman syndrome				
Etiology	**Frequency (%)**	**Genotype (CN)**	**SNP microarray expected result**	**MS-MLPA expected methylation status**	**Etiology**	**Frequency (%)**	**Genotype (CN)**	**SNP microarray expected result**	**MS-MLPA expected methylation status**
Deletion of paternal 15q11.2 (PWCR)	65–75	M/– (1)	Deletion	SNRPN, MAGEL2 100% (GOM)	Deletion of maternal 15q11.2 (UBE3A)	65–75	–/P (1)	Deletion	SNRPN, MAGEL20% (LOM)
Maternal UPD15	20–30	M/M (2)	LOH	SNRPN, MAGEL2 100% (GOM)	UBE3A mutations	11	M/P (2)	Normal	LOM
IC epimutations	2	M/P (2)	Normal	GOM	Paternal UPD15	3–7	P/P (2)	LOH	SNRPN, MAGEL20% (LOM)
IC deletions	0.50		Deletion	GOM	IC epimutations or deletions	3		Normal/deletion	LOM
Normal		M/P (2)	Normal	50%	Normal		M/P (2)	Normal	50%
Beckwith–Wiedemann syndrome					**Russell–Silver syndrome**				
Etiology	**Frequency (%)**	**Genotype (CN)**	**SNP microarray expected result**	**MS-MLPA expected methylation status**	**Etiology**	**Frequency (%)**	**Genotype (CN)**	**SNP microarray expected result**	**MS-MLPA expected methylation status**
LOM of maternal IC2	50	M/P (2)	Normal	IC1: 50%, IC2: LOM	LOM of IC1	35–50	M/P (2)	Normal	IC1:LOM
Paternal UPD11	20	P/P (2)	LOH	IC1:GOM, IC2:LOM	Maternal UPD7	7–10	M/M (2)	LOH Chr7	
Deletion of maternal 11p15.5	9	–/P (1)	Deletion	IC1:GOM, IC2:LOM	Duplication of maternal 11p15.5	Unknown		Duplication	IC1:LOM
GOM of maternal IC1	5	M/P (2)	Normal	IC1:GOM, IC2: 50%					
CDKN1C mutations	5	M/P (2)	Normal						
Normal		M/P (2)	Normal	IC1:50%, IC2: 50%	Normal		M/P (2)	Normal	IC1:50%, IC2: 50%

Abbreviations: CN, copy number; GOM, gain of methylation; IC1, imprinting center 1 containing *IGF2* and *H19* genes; IC2, imprinting center 2 *CDKN1C* and *KCNQ1OT1* genes; LOH, loss of heterozygosity; LOM, loss of methylation; M, maternal; MS-MLPA, methylation-specific Multiplex Ligation-dependent Probe Amplification; P, paternal; SNP, single nucleotide polymorphism; SNRPN, small nuclear ribonucleoprotein polypeptide N; UPD, unipaternal dysomy.

used. Both kits use the same technique. Initially, DNA samples were denaturated and MS-MLPA probes were hybridized to the sample DNA. After the hybridization, the reaction was split into two tubes, one for regular MLPA for copy number analysis (ligation and PCR) and one for the MS-MLPA (ligation, methylation-sensitive HhaI restriction, and PCR) for methylation analysis. The amplification products were separated off by capillary electrophoresis (Applied Biosystems 3500 Genetic Analyzer, Applied Biosystems, United States) and the acquired data were analyzed (Coffalyser.Net MLPA analysis software, http://coffalyser.wordpress.com/).

For both kits (ME28 and ME30), initially copy number analysis was done. The dosage quotient of the probes was analyzed according to the manufacturers recomendations.[10,11] Digestion of control probes (340 and 460 nt probes in ME28, 355nt probe in ME30; 0% methylated in normal DNA) were checked to confirm restriction endonuclease digestion.[10,11]

For methylation analysis of the 15q11 locus (ME28 kit), the six MS-MLPA probes detecting sequences in the *SNRPN*, *MAGEL2*, and *UBE3A* genes and for methylation analysis of the 11p15.5 locus (ME30 Kit), the nine MS-MLPA probes targeting the *H19*, *KCNQ1OT1*, and *IGF2* genes were analyzed. Interpretations were done according to the manufacturers' recommendations.[10,11]

Results

SNP-Microarray and MS-MLPA Results

In a total number of seven patients, LOH covering 15q11.2 and 11p15.5 critical loci was detected. While five were associated with the 15q11.2 locus, and two were associated with the 11p15.5 locus. None of the seven patients had CNVs regarding their clinical presentations.

Two (28.6%) of these seven patients showed aberrant methylation in MS-MLPA: Patient 1 was a 1-year-old girl with hypotonia and microcephaly. She also had dysmorphic features such as almond-shaped eyes and small hands. The phenotype of the patient was clinically consistent with PWS. She had 16.340 kbp LOH at 15q11.2 region. Results of the MS-MLPA study demonstrated that the four *SNRPN* (peak ratios: 0.85/0.97/0.87/0.86) and one *MAGEL2* (peak ratio: 0.98) methylation-specific probes were showing GOM (~100%) instead of the normal 50% methylation value. These results were suggesting the existence of maternal UPD at 15q11.2 region.

Patient 7 was a 1-year-old boy with hemihypertrophy being evaluated for BWS. He had 4.475 kbp LOH at 11p15.5 region. In the MS-MLPA study, the four *H19* (peak ratios: 0.88/0.78/0.82/0.76) methylation-specific probes were showing GOM and the four *KCNQ1OT1* (peak ratios: 0.27/0.22/0.33/0.25) methylation-specific probes were showing LOM (normal: ~0.5). These results were suggesting the existence of paternal UPD at 11p15.5 region.

SNP microarray results are demonstrated in ►**Fig. 1**. Detailed definition of the detected LOH regions and MS-MLPA results is listed in ►**Table 2**. MS-MLPA results are demonstrated in ►**Fig. 2**.

Discussion

Genomic imprinting is a critical procedure for normal development; however, some pathogenic processes (mutations in imprinted genes, CNVs covering imprinted genes, UPD, and epimutations) alter normal imprinting.[3] Although the nature of these mechanisms is very different from each other, their consequent effect is usually the disruption of the normal methylation pattern.[12]

Until now, more than 100 genes have been associated with genomic imprinting. Among these, the abnormal imprinting of 15q11.2 and 11p15.5 loci has been associated with the four well-known imprinting disorders: PWS, AS, BWS, and RSS. In the diagnosis of these disorders, DNA methylation analysis is recommended as the first tier test, since it will detect more than 99% of individuals with PWS and 80% of individuals with AS.[6,13] MS-MLPA is the prominent method, since it detects both methylation status and copy number changes, it is well optimized, it does not require special infrastructure, and it is relatively easy to carry out.[6,14] However, as the SNP microarray became widely used, it has also started to be used in this group of patients, due to the facts that it was also recommended as the first test in DD and CA, as it was giving information about the whole genome, and was able to detect LOH and mosaicisms.[9]

In our department, all patients with DD and CA are initially tested with SNP microarray; thus, we have found the chance to compare the SNP microarray LOH results with MS-MLPA results. One (20%) of the five, 15q11.2 LOH detected patient samples and one (50%) of the two, 11p15.5 LOH detected patient samples showed aberrant methylation in MS-MLPA. These results confirmed the diagnosis of PWS in Patient 1 (maternal UPD at 15q11.2) and the diagnosis of BWS in Patient 7 (paternal UPD at 11p15.5). Our results have showed a low-level correlation (two out of seven; 28.6%) between SNP microarray LOH results and MS-MLPA methylation results.

The efficiency of SNP microarray in the detection of LOH/UPD was previously investigated in the literature. In 2013, Tucker et al reported their results in validating CMA for the identification of UPD. They have reported that CMA had detected 9 (69%) of 13 cases with iso/heterodisomy and all four undetected cases showed an involvement of chromosome 15. They suggested that CMA would fail to identify cases of complete hetero-UPD and cannot exclude the diagnosis of imprinting disorders.[15] Liu et al reported their four patients with imprinting disorders (two had microdeletions). They used SNP microarray and MS-MLPA to validate these variations. One patient with a mosaic UPD in the 11p15.4 region (50%) was detected by SNP microarray. They have suggested that SNP microarray is an efficient alternative method to estimate the sizes and mosaicism rates of CNVs and most types of UPDs.[16] Wang et al investigated the role of regions of homozygosity (ROHs/LOHs) in clinical utility. They have reported that all seven cases (100%) with ROH of whole chromosome 15, and five of eight cases (63%) with segmental ROH 15 were confirmed to be clinically PWS or AS by methylation study. They have suggested that, if the size of an ROH is smaller than 25% of the whole chromosome, it may be

Fig. 1 Single nucleotide polymorphism microarray loss of heterozygosity regions of the patients.

coincidental.[17] Santoro et al examined the efficiency of SNP microarray in identifying ROH in patients with PWS. They have determined that SNP microarray detected an ROH (>10 Mb) in 7 (58%) of 12 patients with previously confirmed PWS by methylation analysis (UPD). They suggested that SNP microarray is a useful diagnostic test in a hypotonic infant with suspected PWS.

When we compare our detection rates (28.6%) with the ones in the literature (ranging from 50 to 69%), it is clear that the rates in our study are quite low. This may be related to the following reasons:

1. In our study, the SNP microarray kit used was CytoScan Optima Array Kit from Affymetrix and this kit contains 148,000 SNP markers. The ChAS analysis software uses the null hypothesis to determine LOHs. If there are not sufficient number of heterozygous calls, then the decision is made in favor of LOH.[18] Thus, this number of SNP markers, design of

Table 2 SNP microarray and MS-MLPA results

Patient	Phenotype	SNP microarray LOH region	Size and number of markers in LOH region	SNP microarray LOH genes	MS-MLPA								
					MS-MLPA Probemix ME028 PWS/AS								
					SNRPN 142nt	SNRPN 178nt	SNRPN 190nt	SNRPN 250nt	MAGEL2 232nt	UBE3A 184nt			
1	Hypotonia, microcephaly, dysmorphism	arr[hg19] 15q11.2q14 (22,809,979–39,150,467) hmz	16.340kbp/865 markers	*TUBGCP5*, *CYFIP1*, *NIPA2*, *NIPA1*, ***MKRN3***, ***MAGEL2***, ***NDN***, ***PWRN2***, ***PWRN1***, ***NPAP1***, ***SNRPN***, ***PWAR5***, ***SNORD116–1***, ***IPW***, ***PWAR1***, ***SNORD115–1***, ***UBE3A***, ***ATP10A***, *GABRB3*, *GABRA5*, *GABRG3*, *OCA2*, *HERC2*, *APBA2*, *NDNL2*, *TJP1*, *CHRFAM7A*, *FAN1*, *TRPM1*, *MIR211*, *KLF13*, *OTUD7A*, *CHRNA7*, *ARHGAP11A*, *SCG5*, *GREM1*, *FMN1*, *RYR3*, *AVEN*, *CHRM5*, *SLC12A6*, *NOP10*, *NUTM1*, *LPCAT4*, *GOLGA8B*, *GJD2*, *ACTC1*, *AQR*, *MEIS2*, *SPRED1*, *RASGRP1*	**0.86 ↑**	**0.97 ↑**	**0.87 ↑**	**0.85 ↑**	**0.98 ↑**	0.01			
2	Dysmorphism	arr[hg19] 15q11.2q13.1 (22,809,979–30,168,981) hmz	7.359 kbp/374 markers	*TUBGCP5*, *CYFIP1*, *NIPA2*, *NIPA1*, ***MKRN3***, ***MAGEL2***, ***NDN***, ***PWRN2***, ***PWRN1***, ***NPAP1***, ***SNRPN***, ***PWAR5***, ***SNORD116–1***, ***IPW***, ***PWAR1***, ***SNORD115–1***, ***UBE3A***, ***ATP10A***, *GABRB3*, *GABRA5*, *GABRG3*, *OCA2*, *HERC2*, *APBA2*, *NDNL2*, *TJP1*	0.5	0.5	0.42	0.45	0.53	0.03			
3	Developmental disability	arr[hg19] 15q11.2q14 (22,809,979–39,390,253) hmz	16.580 kbp/880 markers	*TUBGCP5*, *CYFIP1*, *NIPA2*, *NIPA1*, ***MKRN3***, ***MAGEL2***, ***NDN***, ***PWRN2***, ***PWRN1***, ***NPAP1***, ***SNRPN***, ***PWAR5***, ***SNORD116–1***, ***IPW***, ***PWAR1***, ***SNORD115–1***, ***UBE3A***, ***ATP10A***, *GABRB3*, *GABRA5*, *GABRG3*, *OCA2*, *HERC2*, *APBA2*, *NDNL2*, *TJP1*, *CHRFAM7A*, *FAN1*, *TRPM1*, *MIR211*, *KLF13*, *OTUD7A*, *CHRNA7*, *ARHGAP11A*, *SCG5*, *GREM1*, *FMN1*, *RYR3*, *AVEN*, *CHRM5*, *SLC12A6*, *NOP10*, *NUTM1*, *LPCAT4*, *GOLGA8B*, *GJD2*, *ACTC1*, *AQR*, *MEIS2*, *SPRED1*, *RASGRP1*	0.53	0.52	0.54	0.49	0.59	0.01			

Table 2 (*Continued*)

Patient	Phenotype	SNP microarray LOH region	Size and number of markers in LOH region	SNP microarray LOH genes	MS-MLPA								
					MS-MLPA Probemix ME028 PWS/AS								
					SNRPN 142nt	SNRPN 178nt	SNRPN 190nt	SNRPN 250nt	MAGEL2 232nt	UBE3A 184nt			
4	Developmental disability	arr[hg19] 15q11.2q13.3 (22,809,979–31,791,463) hmz	8.981 kbp/421 markers	*TUBGCP5, CYFIP1, NIPA2, NIPA1,* ***MKRN3, MAGEL2, NDN, PWRN2, PWRN1, NPAP1, SNRPN, PWAR5, SNORD116–1, IPW, PWAR1, SNORD115–1, UBE3A, ATP10A****, GABRB3, GABRA5, GABRG3, OCA2, HERC2, APBA2, NDNL2, TJP1, CHRFAM7A, FAN1, TRPM1, MIR211, KLF13, OTUD7A*	0.49	0.56	0.51	0.56	0.55	0.01			
5	Developmental disability	arr[hg19] 15q11.2q14 (22,809,979–38,834,568) hmz	16.024 kbp/847 markers	*TUBGCP5, CYFIP1, NIPA2, NIPA1,* ***MKRN3, MAGEL2, NDN, PWRN2, PWRN1, NPAP1, SNRPN, PWAR5, SNORD116–1, IPW, PWAR1, SNORD115–1, UBE3A, ATP10A****, GABRB3, GABRA5, GABRG3, OCA2, HERC2, APBA2, NDNL2, TJP1, CHRFAM7A, FAN1, TRPM1, MIR211, KLF13, OTUD7A, CHRNA7, ARHGAP11A, SCG5, GREM1, FMN1, RYR3, AVEN, CHRM5, SLC12A6, NOP10, NUTM1, LPCAT4, GOLGA8B, GJD2, ACTC1, AQR, MEIS2, SPRED1, RASGRP1*	0.54	0.52	0.57	0.51	0.55	0.01			
6	Hemihypertrophy	arr[hg19] 11p15.5p15.5 (243,852–2,101,804)	1.857 kbp/62 markers	*PSMD13, NLRP6, IFITM5, IFITM2, IFITM1, IFITM3, PKP3, SIGIRR, PTDSS2, RNH1, HRAS, RASSF7, MIR210, PHRF1, IRF7, CDHR5, SCT, DRD4, DEAF1, EPS8L2, TALDO1, CEND1, SLC25A22, PIDD, RPLP2, PNPLA2, EFCAB4A, CD151, POLR2L, TSPAN4, AP2A2, MUC6, MUC2, MUC5B, TOLLIP, BRSK2, MOB2, DUSP8, KRTAP5–1, CTSD, SYT8, TNNI2, LSP1, TNNT3, MRPL23,* ***H19***	0.55	0.61	0.53	0.52	0.53	0.55	0.6	0.57	0.04

Table 2 (*Continued*)

Patient	Phenotype	SNP microarray LOH region	Size and number of markers in LOH region	SNP microarray LOH genes	MS-MLPA probemix ME030 BWS/RSS								
					H19 135nt	H19 184nt	H19 238nt	H19 301nt	KCNQ1OT1 141nt	KCNQ1OT1 166nt	KCNQ1OT1 274nt	KCNQ1OT1 393nt	IGF2 171nt
7	Hemihyper-trophy	arr[hg19] 11p15.5p15.4 (230,750–4,706,037) hmz	4.475 kbp/214 markers	*SIRT3, PSMD13, NLRP6, IFITM5, IFITM2, IFITM1, IFITM3, PKP3, SIGIRR, PTDSS2, RNH1, HRAS, RASSF7, MIR210, PHRF1, IRF7, CDHR5, SCT, DRD4, DEAF1, EPS8L2, TALDO1, CEND1, SLC25A22, PIDD, RPLP2, PNPLA2, EFCAB4A, CD151, POLR2L, TSPAN4, AP2A2, MUC6, MUC2, MUC5B, TOLLIP, BRSK2, MOB2, DUSP8, KRTAP5–1, CTSD, SYT8, TNNI2, LSP1, TNNT3, MRPL23,* ***H19****,* ***IGF2****, IGF2-AS, INS, TH, ASCL2, C11orf21, TSPAN32, CD81, TSSC4, TRPM5,* ***KCNQ1****,* ***KCNQ1OT1****,* ***KCNQ1DN****,* ***CDKN1C****, SLC22A18AS, SLC22A18, PHLDA2, NAP1L4, CARS, OSBPL5, MRGPRG, MRGPRE, ZNF195, ART5, ART1, CHRNA10, NUP98, PGAP2, RHOG, STIM1, RRM1, TRIM21, TRIM68, OR51E1, OR51E2*	**0.88 ↑**	**0.78 ↑**	**0.82 ↑**	**0.76 ↑**	**0.27 ↓**	**0.22 ↓**	**0.33 ↓**	**0.25 ↓**	0.03

Abbreviations: AS, Angelman syndrome; LOH, loss of heterozygosity; LOM, loss of methylation; MS-MLPA, methylation-specific Multiplex Ligation-dependent Probe Amplification; PWS, Prader–Willi syndrome; SNP, single nucleotide polymorphism; SNRPN, small nuclear ribonucleoprotein polypeptide N.
Note: The gene names in bold represent the phenotype-associated imprinted genes.

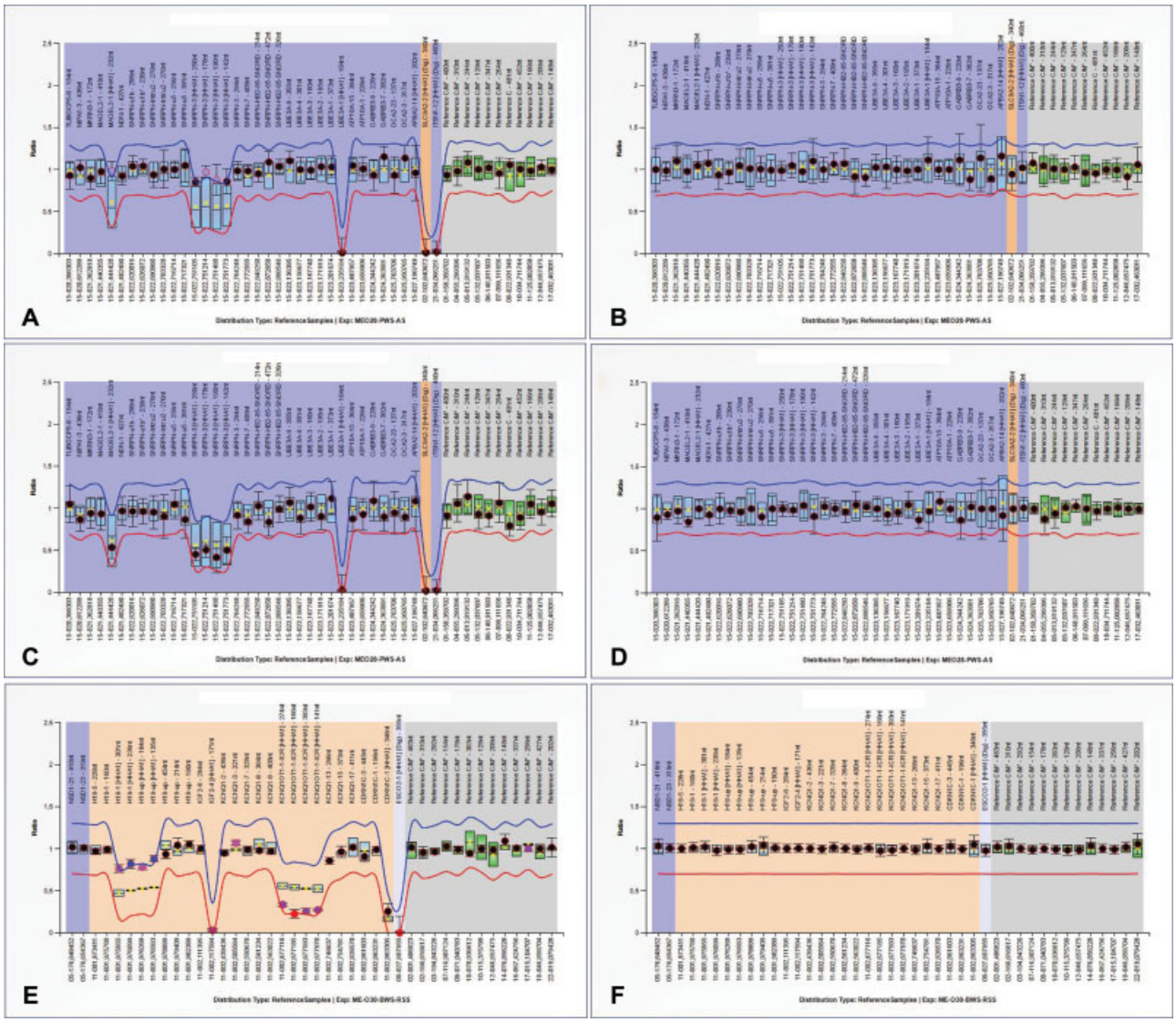

Fig. 2 MS-MLPA results of Patient 1 (15q11.2 MS-MLPA), Patient 2 (15q11.2 MS-MLPA), and Patient 7 (11p15.5 MS-MLPA). (**A**) Peak ratios of HhaI digested Patient 1 sample. The four SNRPN (ratios: 0.85/0.97/0.87/0.86) and one MAGEL2 (ratio: 0.98) methylation-specific probes were showing increased peak ratios (normal: ~0.5). (**B**) Peak ratios of (undigested) Patient 1 sample, showing normal copy number peak ratios. (**C**) Peak ratios of HhaI digested Patient 2 sample, showing normal methylation peak ratios. (**D**) Peak ratios of (undigested) Patient 2 sample, showing normal copy number peak ratios. (**E**) Peak ratios of HhaI digested Patient 7 sample. The four H19 (ratios: 0.88/0.78/0.82/0.76) methylation-specific probes were showing increased peak ratios and the four KCNQ1OT1 (ratios: 0.27/0.22/0.33/0.25) methylation-specific probes were showing decreased peak ratios (normal: ~0.5). (**F**) Peak ratios of (undigested) Patient 7 sample, showing normal copy number peak ratios. MS-MLPA, methylation-specific Multiplex Ligation-dependent Probe Amplification; SNRPN, small nuclear ribonucleoprotein polypeptide N.

the microarray kit, or the software algorithm may not be appropriate to make a more accurate assessment (CytoScan HD used by Wang et al was containing 750,000 SNP markers and the Human SNP Array 6.0 used by Tucker et al was containing 906,600 SNP markers).

2. The detected LOHs of all seven patients in our patient group were segmental LOHs and the average size of the LOHs was 10,230 kbp. As indicated by Wang et al, smaller LOHs tend to be coincidental; thus, they may not be reflections of real UPDs.

Normally, any single SNP at any position can be homozygous; however, if the subsequent homozygous alleles cover a chromosomal region, long contiguous stretches of homozygosity (LCSH) (ROHs or LOHs) come into question. LCSH are frequently detected by SNP microarrays. ROHs/LOHs may arise from parental relatedness, ancestral homozygosity, consanguinity, or UPD; thus, the detected LOHs can be coincidental as well as reflections of real UPDs.[19] There is no clear way to predict the pathogenicity of LCSH without additional molecular evaluations. However, it is known that recombinations scatter these long homozygous segments and they become smaller by generations to generations. Thus, if a long homozygotic stretch is detected, it may be presumed that it may be associated with a real UPD, since it may be a result of a trisomic rescue or monosomic compensation that occurred at that individual's embryonic life.[5,20] There is no clear data on how long an LCSH can actually be associated with UPD; however, Papenhausen

et al tried to determine a threshold value.[20] They have reported that among nine confirmed UPD cases with anticipated LCSH, the smallest one was 13.5 Mb in length. They have suggested that this length was above the average longest LCSH in a set of control patients and was then set as the prospective threshold for reporting possible UPD correlation.[20] Contrary to this suggestion, Iourov et al reported that shorter LCSH at chromosomes 7q21.3, 7q31.2, 11p15.5, and 15p11.2 occurs with a frequency of ~5% in the children with DD, CA, and epilepsy. They have suggested that LCSH that are 2.5 to 10 Mb in size can also be associated with DD, CA, and epilepsy etiology.[19]

Conclusion

As a conclusion, all these data indicate that it is not possible to predict the pathogenic effects of ROHs/LOHs detected in SNP microarray and the probability of their actual association with UPDs, without additional molecular genetic studies.

SNP microarray analysis is a useful tool in the detection of LOH. Furthermore, it has the advantage of giving information about other regions of the genome, determining the parental origin, breakpoints, and the sizes of abnormal fragments and the ratio of mosaicism[8]; however, it should be used with caution, since false-positive or false-negative LOH results can be obtained. Moreover, it must be kept in mind that it may fail to identify hetero-UPD. Although methylation analysis is recommended as the first tier test in the diagnosis of most of the imprinting disorders, combining methylation analysis with SNP microarray analysis, and moreover, using SNP microarray as a first tier test, can enhance our evaluation process. This better understanding of the disease mechanism will improve the quality of genetic counseling as well as preimplantation/prenatal diagnosis planning.

Ethics Approval

The study was not submitted to a research ethics committee. No financial or nonfinancial benefits have been received or will be received from any party related directly or indirectly to the subject of this article. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975 (in its most recently amended version). Informed consent was obtained from all patients included in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this article. All institutional and national guidelines for the care and use of laboratory animals were followed. This article does not contain any studies with human or animal subjects.

Authors' Contributions

All the authors contributed to the design and implementation of the research, analysis of the results, and writing of the article.

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Conflict of Interest

None declared.

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